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**THE BIURET REACTION. IV. (a) A BIURET
SALT OF THE TETRAPEPTIDE TRIGLYCYL-
GLYCINE. (b) A BIURET SALT OF GLYCINE
ANHYDRIDE. (c) THE BARIUM BIURET
SALT OF SUCCINIMIDE**

A DISSERTATION SUBMITTED TO THE
FACULTY OF THE DIVISION OF THE
PHYSICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF CHEMISTRY

1932

By
FRANCIS MILTON PARKER

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[Reprinted from the Journal of the American Chemical Society, 56, 1168 (1934).]

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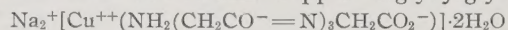


The Biuret Reaction. IV. (a) A Biuret Salt of the Tetrapeptide Triglycylglycine. (b) A Biuret Salt of Glycine Anhydride. (c) The Barium Biuret Salt of Succinimide

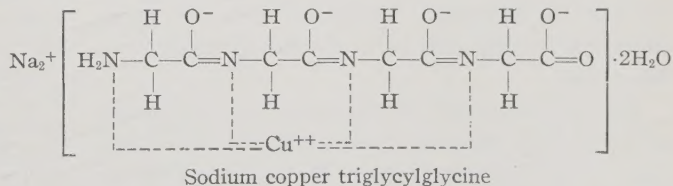
Following the investigation of the biuret reaction of amino acid amides,¹ and in preparation for a study of the biuret reaction of the proteins, the biuret salt of the tetrapeptide triglycylglycine, $\text{NH}_3^+ (\text{CH}_2\text{CONH})_3\text{CH}_2\text{CO}_2^-$ has been isolated and examined.

The synthesis of triglycylglycine was accomplished in a series of reactions described by Fischer.² Glycine was converted into its ester hydrochloride, from which there was obtained glycine anhydride. The anhydride yielded upon hydrolysis and treatment with chloroacetyl chloride in one operation chloroacetylglycylglycine, which was converted by the use of ammonia into diglycylglycine. By treatment of the tripeptide successively with chloroacetyl chloride and ammonia, triglycylglycine was obtained. It was found that liquid ammonia acts more efficiently in the ammonolysis of certain of the chloroacetyl peptides than does the aqueous ammonia recommended by Fischer.

The biuret salt sodium copper triglycylglycine



was prepared by treatment of the tetrapeptide with copper acetate and sodium hydroxide. The analytical data for the salt point to the empirical formula just given. It is of peculiar interest to note that the molecule of the salt contains only one peptide molecule, which furnishes the four basic nitrogen atoms and the four acid hydrogen atoms which have already been found requisite for the occurrence of a number of typical biuret reactions. The molecular weight of the salt has not been ascertained. If its molecular and empirical formulas are identical the structure of the salt may be



(1) Rising and Yang, *J. Biol. Chem.*, **99**, 755 (1933).

(2) E. Fischer, "Untersuchungen über Aminosäuren, Polypeptide und Proteine," Vols. I and II, Verlag von Julius Springer, Berlin, 1906.

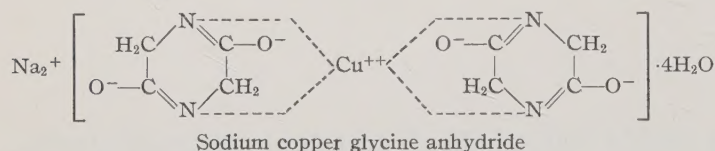
It will be noted that formation of a salt molecule of this structure involves (1) neutralization by alkali of two of the acid groups of an enol

tautomer of the peptide molecule. The other two acid groups serve as the negative radical in the complex salt, as does the sulfate ion in copper ammonium sulfate. The formation of the salt molecule involves (2) complex ion formation of copper with four amine nitrogen atoms.

Incidental to the synthesis of the peptides of the glycine series, the biuret reaction of glycine anhydride was investigated. While glycine itself shows no biuret reaction, its anhydride, $\text{CONHCH}_2\text{CONHCH}_2$ does so readily, behaving as an acyl disubstituted di-acid amide. The biuret salt of the anhydride was isolated, analyzed and its probable empirical formula shown to be



The structure of the salt may be



It is significant that the formula of the compound agrees with that shown previously to be correct for the biuret salts of other di-acid amides, *e. g.*, $\text{Me}_2^+[\text{Cu}(\text{di-acid amide})_2]^- \cdot x\text{H}_2\text{O}$.

It is a curious fact that the first peptide to show the biuret reaction in the glycine series is triglycylglycine. The cause of the failure of many di- and tripeptides to react with cupric ion and alkali should be investigated, since there is no apparent reason for this sharp difference in behavior between the tetrapeptides and their precursors. Di-*l*-alanyl-*l*-alanine is said to show a "weak" biuret reaction and the isolation of its biuret salt is now being attempted.

The pentapeptide tetraglycylglycine has been prepared from chloroacetyltriglycylglycine and liquid ammonia and converted into its biuret salt by Paul E. Wenaas. Analytical data and other facts concerning this salt will be reported at a later time.

For the adequate development of the theory of the biuret reaction there must be uncovered facts regarding the nature of the hypothetical "biuret acids," the parent acids of biuret salts. There should exist, for example, however unstable they may be, the acids $\text{H}_2^+[\text{Cu}(\text{malonamide})_2]^-$, corresponding to the salt $\text{Na}_2^+[\text{Cu}(\text{malonamide})_2]^-$, and $\text{H}_2^+[\text{Cu}(\text{succinimide})_4]^-$, the

parent acid of the salt $\text{K}_2^+[\text{Cu}(\text{succinimide})_4]^-$. The acids should be, in certain instances at least, obtainable from their salts, and it is anticipated that the barium salts may prove particularly suitable for this purpose. Accordingly, the biuret salt barium copper succinimide was prepared, of formula $\text{Ba}^{++}[\text{Cu}^{++}(\text{CH}_2\text{CH}_2\text{CON}=\text{CO}^-)_4] \cdot \text{H}_2\text{O}$.

This salt is comparable in formula and doubtless in structure with potassium copper succinimide, $\text{K}_2^+[\text{Cu}^{++}(\text{CH}_2\text{CH}_2\text{CON}=\text{CO}^-)_4] \cdot 6\text{H}_2\text{O}$.

Experimental

Synthesis of Peptides.—The procedure described by Fischer for the preparation of triglycylglycine was followed faithfully with only one notable change: in the ammonolysis of chloroacetyltriglycylglycine and chloroacetyldiglycylglycine, liquid, rather than aqueous, ammonia was used by us. The tri- and tetrapeptides so obtained were purer and formed in about 8% larger yields than was the case when Fischer used aqueous ammonia.

Sodium Copper Triglycylglycine, $\text{Na}_2\text{CuC}_8\text{H}_{14}\text{N}_4\text{O}_7$.—The salt was prepared as follows: a filtered solution of 1.5 g. of triglycylglycine in 90 cc. of water was treated with 30 cc. of 2% sodium hydroxide solution and sufficient freshly precipitated copper hydroxide so that some of the latter base remained undissolved at the end of the reaction. The red reaction solution was shaken for five minutes and then filtered into 2000 cc. of a 2-1 absolute alcohol-ether mixture. Presently a pink precipitate formed, which was collected, dried at room temperature over phosphorus pentoxide and analyzed.

Anal. Calcd. for $\text{Na}_2\text{CuC}_8\text{H}_{14}\text{N}_4\text{O}_7$: Na, 11.86; Cu, 16.40; C, 24.76; H, 3.64; N, 14.45. Found: Na, 12.39, 12.82; Cu, 16.47, 16.73; C, 24.87, 24.44; H, 4.24, 4.39; N, 14.23, 14.00.

Dehydration of this salt was carried out at 80° and 15 mm. over phosphorus pentoxide. The dehydrated substance was analyzed for nitrogen.

Anal. Calcd. for $\text{Na}_2\text{CuC}_8\text{H}_{10}\text{N}_4\text{O}_5$: N, 15.93. Found: N, 15.64, 15.70.

The analytical data indicate that dehydration of the compound *in vacuo* at 80° causes a loss of two molecules of water. The salt is a deep pink powder which chars at 278° (corr.) and melts with decomposition at 280° (corr.).

Sodium Copper Glycine Anhydride, $\text{Na}_2\text{CuC}_8\text{H}_{16}\text{N}_4\text{O}_8$.—The instability of this salt led to considerable difficulty in its isolation. The method used follows: 1 g. of glycine anhydride and 0.428 g. of freshly precipitated and washed copper hydroxide were suspended together in about 40 cc. of water. A solution of 0.7 g. of carbonate-free sodium hydroxide in 8 cc. of water was brought into this reaction mixture, whereupon a deep blue color developed. After ten minutes all of the anhydride and copper hydroxide had dissolved, and the solution was then concentrated under reduced pressure until its volume was about 17 cc. The

colored liquid was added drop by drop with stirring to a mixture of 700 cc. of absolute ethyl alcohol and 350 cc. of absolute ether, whereupon a deep lavender, finely divided precipitate of sodium copper glycine anhydride formed; this was collected as rapidly as possible on a filter. The extremely hygroscopic nature of the salt made its handling under a protective layer of dry alcohol and ether a necessity. The compound was stable in a desiccator over fresh phosphorus pentoxide.

Anal. Calcd. for $\text{Na}_2\text{CuC}_8\text{H}_{16}\text{N}_4\text{O}_8$: Na, 11.33; Cu, 15.67; C, 23.66; H, 3.97; N, 13.80. Found: Na, 11.48, 11.40; Cu, 14.52, 14.57; C, 24.82, 25.10; H, 4.16, 4.34; N, 13.63, 13.66.

The results of dehydration experiments indicated the presence of four molecules of water in the salt molecule. The substance decomposes when heated above 120° .

Barium Copper Succinimide, $\text{BaCuC}_6\text{H}_8\text{N}_2\text{O}_5$.—The salt was prepared as follows: 50 cc. of a saturated aqueous solution of barium hydroxide was filtered into 250 cc. of 90% alcohol which contained 2.5 g. of succinimide. Then 15 cc. of a saturated aqueous solution of copper acetate was filtered into this mixture, which was shaken meanwhile.

After about two minutes a pink precipitate formed which was collected and dried to constant weight over phosphorus pentoxide at 100° and 15 mm. The analytical data for the dehydrated salt follow:

Anal. Calcd. for $\text{BaCuC}_6\text{H}_8\text{N}_2\text{O}_5$: Ba, 23.10; Cu 10.72; C, 32.37; H, 2.71; N, 9.44. Found: Ba, 23.67, 23.66; Cu, 10.74, 10.61; C, 30.10, 29.92; H, 3.12, 3.05; N, 9.54, 9.37.

The salt, which is a brownish-red powder, melts with decomposition at 257° (corr.).

Summary

1. The sodium biuret salt of the tetrapeptide triglycylglycine, the barium biuret salt of succinimide and the sodium biuret salt of the acyl disubstituted di-acid amide glycine anhydride have been isolated and analyzed.

2. The theory and facts of the biuret reaction as so far developed are in agreement with the character of these salts.



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